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Uptake and Turnover of a Single Injected Dose of I^{131} in Tadpoles of *Rana clamitans*[1,2]

NANCY WEBER KAYE[3] & ELIZABETH E. LE BOURHIS

Department of Zoology, Barnard College, Columbia University, New York

(Text-figures 1-3)

INTRODUCTION

IN 1941 Gorbman & Evans first demonstrated by means of radioautographs that the thyroid of an anuran tadpole, *Hyla regilla,* can accumulate, in organic combination, radioactive iodine placed in the water as iodide. Dent & Hunt (1952), also employing radioautographs, have mapped the radioiodine distribution in *Hyla versicolor*. Later, Hunt & Dent (1957) studied, by quantitative techniques, the radioiodine uptake and turnover in frog tadpoles in whose environmental water radioiodide was placed. Money, Lucas & Rawson (1955) and Saxén *et al.* (1957) have conducted similar studies with tadpoles of *Rana pipiens* and *Xenopus laevis* respectively.

None of the observations on thyroidal radioiodine accumulation in radioiodide-immersed animals gives a clear idea of the metabolism of a single tracer dose of the isotope. Since absorption from the environment must occur in immersed animals, various additional physiologically variable factors are interposed between the thyroid function one wishes to measure and the tracer iodine. It is not possible, with any degree of assurance or precision, to express the thyroidal I^{131} in terms of per cent. uptake of a given dose since the amount of I^{131} which actually enters the animal is difficult to evaluate and varies from one animal to the next. Furthermore, the effect of number and/or size of animals per unit volume of environmental water and of the amount of "carrier" iodide in this water is not easily determined.

For these reasons the present study was undertaken. The purpose of these observations was the determination of the fate of a single dose of carrier-free radioiodide injected into the tadpole.

MATERIALS AND METHODS

Premetamorphic tadpoles of *Rana clamitans* of 6-8 cm. in length with hind legs of less than 6 mm. were used (Taylor & Kollros, 1946). Prior to experimentation they were kept in tap water and fed on *Elodea* and corn meal. During the experimental period the tadpoles were kept in tap water, 7 animals per three liters, at 22° C. They were not fed. Into each animal a single dose of 5 microcuries of carrier-free radioiodine (I^{131}) in a 0.05 cc. volume was injected intraperitoneally, the needle being inserted through the tail musculature.

At time intervals of 2, 10, 20, 40, 72 and 120 hours after injection, 6 of the 7 animals in each group were anesthetized by immersion in 0.1% M.S.-222 (Tricaine-methane-sulfonate, Sandoz). Each tadpole was then dissected into a planned number of parts, each of which was placed in a closed vial containing 1 cc. of 0.7 % NaCl. The weight and the I^{131} content of each part was recorded so that the localized concentration of iodine could be followed over a period of time. Measurements of radioactivity were made in a well-type gamma ray scintillation counter (and were corrected for physical decay of the isotope).

RESULTS

Per cent. uptake of the injected I^{131} was calculated for the total animal, thyroid and eyes.

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Concentration was calculated as per cent. uptake per mg. of tissue × 100 (for convenience) for the thyroid, eyes, ventral white skin, tail (both skin and musculature), carcass of head, dark skin of carcass, coiled intestine and remaining viscera. Thyroids were dissected out under the binocular microscope. The small size of the glands obliged us to include small pieces of cartilage, which were necessarily weighed along with the glands.

In Text-fig. 1 the per cent. uptake curve of the eyes, as a typical body tissue, reaches a peak at about the same time as the per cent. uptake curve of the whole animal, whereas the thyroid curve reaches its peak at 72 hours.

Although total thyroid uptake is low, the concentration per mg. of tissue is exceedingly high as compared with the other body tissues in Text-fig. 2.

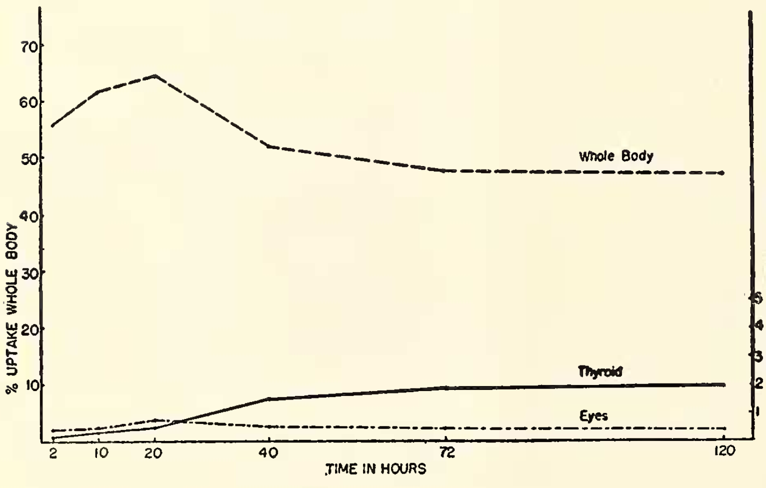
It is interesting to note in Text-fig. 3 that the curves of I^{131} concentration for the various body tissues fall into three groups: tail, eyes and carcass of head in one group; ventral white skin and dark skin of carcass in a second group; and coiled intestine and remaining viscera in a third group.

Discussion

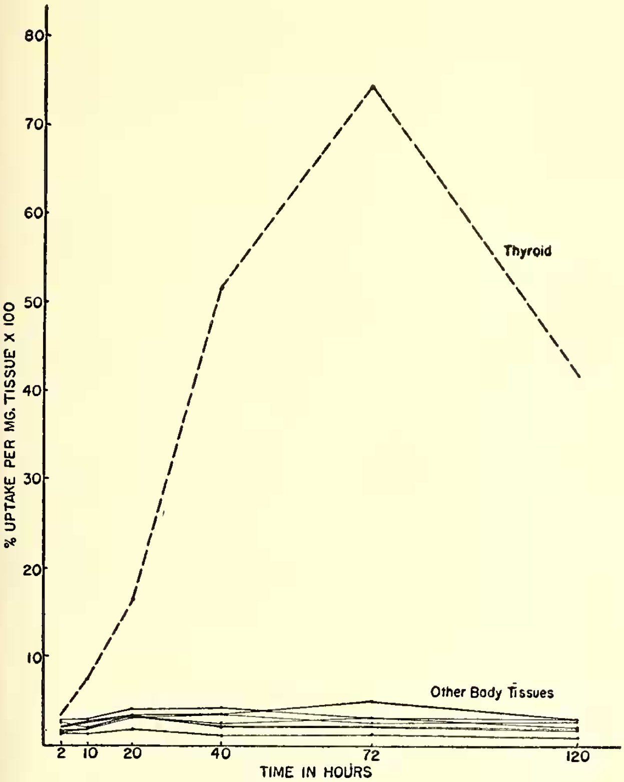
In this study iodine accumulation by the thyroid slowly reached a level of 2 % of the injected dose at 72 hours and remained at this value until the end of the experiment. This level in the thyroid is reached only after the values for the other body tissues have decreased, as indicated by the curve for the pair of eyes in Text-fig. 1. Very possibly the thyroid continues to take up radioiodine from the blood stream after the iodine has been distributed throughout the body. Unpublished data of Gorbman & Dundee show that *Eurycea*, a neotenic form of salamander, also has a low iodine turnover, so that at the end of 7 days the thyroid still contains about 50 % of its peak value of 2.5 % achieved at about 24 hours.

Text-fig. 2 clearly shows that the thyroid is the most potent of all tissues in concentrating radioiodine. Since the dissected thyroid piece includes the weight of the thyroid, as well as the cartilage upon which it rests, the values obtained for concentration are considerably lower than the real values. The peak for thyroid, which is at least 7 times greater than any other value, comes at a time when the majority of other body tissues have reached an equilibrium (Text-fig. 2). Other experiments with anuran tadpoles have not provided information concerning the relative concentrations of iodine in the thyroid and the remainder of the body. The chemical form of the thyroidal iodine in tadpoles is as yet unknown since chromatographic studies have not been done. However, radioautographs indicate that it is bound in an organic form (Dent & Hunt, 1952).

Money, Lucas & Rawson (1955), using an alternative technique of immersing *Rana pipiens* tadpoles of different ages for a definite period of time in a solution of I^{131}, find a value of close to 10-20 % of environmental I^{131} localized in the block of thyroid tissue at the end of five days (MLR Fig. 2). When they injected tadpoles lower thyroidal I^{131} accumulation was found (5-10 %), which still exceeded the values we have observed in *Rana clamitans*. Such data illustrate the differences to be found between single administrations of tracer I^{131} and continuous absorption from the environment. The fact that continuous absorption varies with stage and therefore cannot be used as a standard value is shown also in the work of Saxén *et al.* (1957).

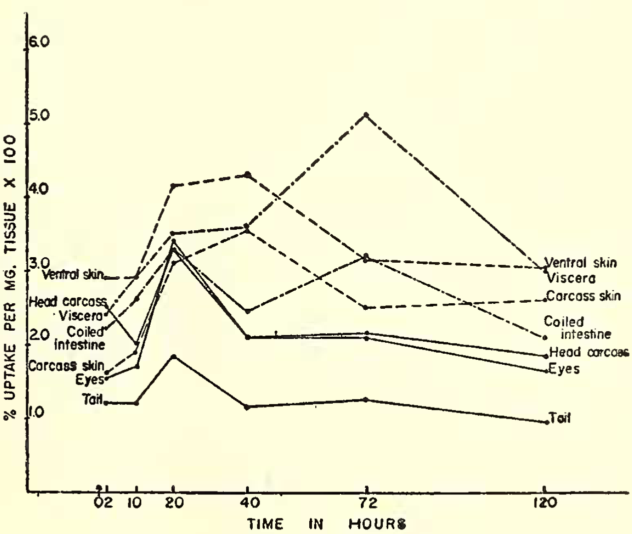


Text-fig. 1. Uptake for whole body, thyroid and eyes. Each point on the curve represents the average of determinations done on six animals.



TEXT-FIG. 2. Concentration of I^{131} in the thyroid and other body tissues. Concentration calculated as per cent. uptake per mg. tissue × 100. Average values determined the same as in Text-fig. 1.

The apparently continued increase in total body I^{131} to 65 % at 20 hours after injection (Text-fig. 1) is at first difficult to interpret. The only apparent explanation is that up to that time a considerable part of the injected dose remained unabsorbed in the body cavity and/or blood stream of the tadpole. The whole body value of the administered dose was found by summing the values obtained for individual body parts. After 20 hours the amount of radioiodine decreases gradually to 47 % and appears to maintain itself at that level. This seems to indicate that extrathyroidal areas, which retain a considerable amount of the injected dose, also have a low turnover. These data agree, in general, with those provided for *Rana pipiens* tadpoles



TEXT-FIG. 3. Concentration of I^{131} in the various body tissues other than thyroid. Average determinations the same as in Text-fig. 1.

by Money, Lucas & Rawson (1955).

Data obtained by Hunt & Dent (1957), who administered I^{131} by a short period of immersion in I^{131} solutions, indicate that after 2 days the total amount of iodine absorbed in *Hyla versicolor* tadpoles decreases from approximately 50 % to 10 % whereas our values during the same period remain near 47 %.

Dent & Hunt in 1952 pursued a study of the I^{131} distribution by radioautographing sections of various tissues. However, their study was not primarily quantitative. By actually measuring the fraction of the administered dose accumulated in various parts of the body by means of a well-type gamma ray scintillation counter, our study has allowed a quantitative description of the distribution of the radioiodine.

It is interesting to note that the curves for the body tissues appear to fall into three sets of parallel curves (Text-fig. 3). The group composed of ventral white skin and dark skin of carcass, unexpectedly, shows higher values for the ventral white skin, which would be expected to contain much less tyrosine. Dent & Hunt (1952) found a denser radioautograph in the region of the skin. They attributed this to the binding of iodine by the tyrosine of the melanin pigment. The tail, with less skin and more musculo-skeletal tissue per unit weight, contained much lower iodine concentration, indicating that such tissue probably does not serve as a storage area for iodine. Both viscera and coiled intestine continue to concentrate I^{131} even at a time when the other tissues seem to be reaching an equilibrium. Entero-hepatic recirculation is an important mode of excretion of thyroid hormones through the liver and intestine. The higher values for these organs may be a reflection of such excretion, since the peaks occur at the same time as the thyroidal peak. This may be taken to indicate that the thyroid in the tadpole does have a turnover, although a low one.

Our experimental data indicate much individual variation, as also shown by both Hunt & Dent (1957) and Money, Lucas & Rawson (1955). Because each group of animals was kept in a large volume of water, the possibility of I^{131} reabsorption is negligible. Hunt & Dent (1957) also feel that the concentration of iodine in the water and the amount of food ingested by the tadpole do not effect the release of iodine. An extension of the experimental period beyond five days would have been desirable.

Acknowledgements

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Summary

1. Per cent. uptake of injected I^{131} was calculated for the whole body, thyroid and eyes of premetamorphic *Rana clamitans.*
2. Distribution of iodine was studied by comparison of I^{131} concentration in the thyroid to the concentration of I^{131} in the other body tissues over a period of five days.
3. Total body per cent. uptake of I^{131} reaches a peak of 65 % at 20 hours and decreases to an equilibrium of 47 % at 72 hours.
4. The fraction of administered dose of I^{131} accumulated by the thyroid reaches a maximum of approximately 2 % at 72 hours.

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